Seizure 30 (2015) 6–13

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Reduction of epileptiform activity through local valproate-implants in a rat neocortical epilepsy model

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ARTICLE INFO

Article history:
Received 12 January 2015
Received in revised form 29 April 2015
Accepted 1 May 2015

Keywords:
Epilepsy
Animal model
Tetanus toxin
Cobalt
Valproate

ABSTRACT

Purpose: Pharmacotherapy of epilepsies is limited due to low concentrations at epileptogenic foci, side effects of high systemic doses and that some potentially efficient substances do not pass the blood–brain barrier. To overcome these limitations, we tested the efficacy of local valproate (VPA)-containing polymer implants in a model of necocortical injected tetanus toxin (TeT) in the rat.
Methods: Tetanus toxin was injected intracortically and cobalt (II) chloride ($CoCl_2$) was applied on the cortical surface. Video-electrocorticography recordings with intracortical electrodes were performed. VPA-containing polymers were implanted above the cortical focus. Antiepileptic effects were evaluated as reductions of epileptiform potentials (EPs) per hour in comparison to saline (NaCl)-containing polymer implants.
Results: Triple 50 ng TeT injections plus $CoCl_2$ application (20/10 mg) showed consistent EPs. NaCl-implanted animals ($n = 6$) showed a mean of 10.5 EPs/h after the first week, the EP frequency increased to 53.5 EPs/h after the second week. VPA-implant animals ($n = 5$) showed a reduction in EP frequency from 71.6 to 4.8 EPs/h after the second week. The EP frequency after the second week was higher in the NaCl-implanted animals than in the VPA-implanted ($p = 0.0303$). The mean EPs/h increase in NaCl-implanted rats (+42.9 EPs/h) was different ($p = 0.0087$) from the mean EPs/h decrease in VPA-implanted rats (−66.8 EPs/h).
Conclusion: Despite former publications no clear seizures could be reproduced but it was possible to establish focal EPs, which proved to be a reliable marker for epileptic activity. Local antiepileptic therapy with VPA has shown efficacy in decreasing EP frequency.

1. Introduction

It is well known that approximately 0.5–1% of the worldwide population suffers from epilepsy [1,2]. About 20–30% of those epilepsies are refractory to pharmacological treatment [1,3,4]. A high percentage of these pharmacoresistant epilepsies are caused by acquired or maldevelopmental cortical lesions. In these cases the development of new antiepileptic drugs (AEDs) has not shown a convincing breakthrough [5]. For many of those patients the surgical removal of these lesions improves the epileptic outcome [6]. However, if the epileptogenic lesion or focus is located in an eloquent brain area, surgery might result in a severe neurological deficit. For these patients, local antiepileptic pharmacotherapy with AED-containing implants could be a treatment option if experimental studies show an effect on epileptic activity.

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http://dx.doi.org/10.1016/j.seizure.2015.05.002
1059-1311/

Most AEDs can suppress any epileptiform discharges, if the drug is concentrated highly enough. However, in systemic pharmacotherapy, systemic adverse effects arising outside of the epileptic focus may prevent sufficiently high AED concentrations in the focus. Several drugs with potential antiepileptic properties cannot reach the epileptogenic brain area because they do not pass the blood–brain barrier [7,8]. In addition, pharmacoresistant epilepsies are often characterized by multiple drug transporters, which prevent a sufficient AED concentration in the focus [9,10]. In contrast, local application of AEDs would advantageously reduce systemic side effects, bypass the blood–brain barrier and diminish the effect of multiple drug transporters by higher local AED concentrations [11,12].

It was shown that intracerebral or intraventricular application of AEDs as polymer implants or pump systems can reduce seizure frequency or severity [13–16]. In a rat model with intracortical tetanus toxin (TeT) injection Nilsen et al. [17] showed ictal and interictal activity which was quantified by electrocorticography (ECoG) and electromyography (EMG). Thus, these authors demonstrated this model to be potentially suitable for measuring effects of a local antiepileptic therapy. In further experiments, the same group then described an antiepileptic effect of locally applied gap-junction blockers [18].

The aim of the present study was to reproduce the described neocortical TeT-injection epilepsy model by Nilsen et al. [17,18] and establish an ECoG-based quantification method for measuring the epileptic activity in this model. If no stable, quantifiable, epileptic activity could be established, this model should be improved by applying more TeT or multiple TeT-injections; or even combine this model with another neocortical model, as the cobalt(II) chloride ($CoCl_2$)-cortical application. Finally, if a stable, quantifiable, epileptic focus would be established, it could prove a potential therapeutic effect of locally applied valproate (VPA)-containing biodegradable polycaprolactone (PCL)-implants.

2. Materials and methods

Animal experiments were carried out according to the European Community Council Directive (86/609/EEC) and the Declaration of Helsinki and were approved by the local government representative (Regierungspräsidium Baden-Wuerttemberg, Freiburg, registration no.: 35/9185.81/G-09/10). Sixty-three male Sprague-Dawley rats (Animal facility of the University Medical Center Freiburg) at a mean age of approximately two and a half months were included. Four different treatments were performed: no toxin, single injection of 50–200 ng TeT, triple injections of 50 ng TeT or triple injections of 50 ng TeT plus either 20 mg (initially) or 10 mg (afterwards) $CoCl_2$.

2.1. Surgery

Surgery was conducted upon sterile conditions. Sprague-Dawley rats were anesthetized with 1.5–2.5% vol. isoflurane and placed in a stereotactic frame (David Kopf instruments, Tujunga, CA). Borehole trephinations were performed (Proxxon, Niersbach, Germany) and the dura mater was perforated with a needle. Injections were performed over a stainless steel cannula (outer diameter, 0.28 mm), connected to a 2 μL microsyringe (Hamilton, Carl Roth GmbH and Co. KG, Karlsruhe, Germany) *via* PE-20 tubing with a micropump (World Precision Instruments, Sarasota, USA). For ECoG recordings, teflon-coated silver electrodes (diameter = 0.38 mm, World Precision Instruments, Sarasota, USA) with de-insulated endings were utilized.

In rats without injection, two ECoG electrodes were placed into the M1 region of the right hemisphere (coordinates in relation to the bregma; [19]): no. 1: anteroposterior (AP) +1.1 mm, mediolateral (ML) −2.5 mm, dorsoventral (DV) −1.0 mm; no. 2: AP +1.1 mm, ML −1.5 mm, DV −1.0 mm. EMG electrodes were inserted into the right and left temporal muscle. The reference and grounding electrode were initially placed into the cerebellum, later placed intraosseously by attachment to implanted screws, which were positioned over the right and left parietal cranium, serving for additional stability. All electrodes were connected to a plug connector, which was embedded in dental cement together with the screws (Paladur®, Heraeus, Hanau, Germany). All animals in this investigation received 5 mL 0.9% saline (NaCl) solution subcutaneously during surgery for fluid replacement and 0.05 mg/kg bodyweight buprenorphine subcutaneously after surgery to prevent postoperative pain.

In rats receiving a single injection of 50–200 ng TeT, the toxin was injected into the M1 area of the right hemisphere (AP +1.1 mm, ML −2.5 mm; DV −1.0 mm) over a 5-min period. Fifty nanogram TeT were solved in 0.5 μL sterile distilled water with 2% bovine serum albumin resulting in a working concentration of 100 ng/μL. In the case of Evans blue-injection, the toxin was solved in 0.25 μL Evans blue solution and 0.25 μL sterile distilled water with 2% bovine serum albumin. To ensure appropriate toxin diffusion 10 min had to elapse after injection. Then the ECoG and EMG electrodes were positioned as described above.

For rats with triple injections of 50 ng TeT, the additional boreholes were AP +2.1 mm, ML −3.5 mm and AP +0.1 mm, ML −3.5 mm. Each injection was performed over a 10-min period to ensure toxin diffusion. Electrode positioning followed as described above.

For rats with triple injections of 50 ng TeT plus 20 mg or 10 mg $CoCl_2$, a hole (5 mm diameter) was carefully drilled with a mill in the skull above the right primary motor cortex (anterior to the bregma, with edges 1 mm lateral to the midline and bordering the coronal suture; Fig. 1A). Drilling was performed upon steady saline cooling to avoid heat. The dura mater was removed with a sharp miniaturized hook. TeT (50 ng) was injected at injection sites described for the animals with triple injections (Fig. 1B). After the injections, 20 or 10 mg crystalline $CoCl_2 \cdot 6H_2O$ was applied onto the cortex. Afterwards, a biodegradable PCL-implant containing either 0.9% NaCl or VPA (10%, w/w each) was placed on top. Finally, the electrodes and the lesion site were covered with cyanoacrylate (Fig. 1D), that did not get in touch with the cortical surface. Production and release kinetics of the implants were described in detail by Kammerer et al. [20]. Rats treated with TeT or $CoCl_2$ showed an increased irritability to noise, physical contact, toward other rats or the hand of the examiner. In addition, they showed an increased aggression. These signs were also described by Brener et al. [21] and Liang et al. [22].

2.2. Recordings, data analysis and histology

In all animals a combined ECoG/EMG-video-monitoring was performed. Recordings started two days after surgery at the earliest to allow recovery and lasted between 90 min and 6 h each. After amplification, data were acquired using a bandpass filter (1 Hz to 5 kHz) and sampled by CED Spike 2 software version 5 (Cambridge Electronic Design Ltd. Cambridge, U.K.). The recordings were analyzed by blinded analyzers, *i.e.* they did not know which treatment the animal had received. The analysis focused on qualitative and quantitative evaluation of both interictal and ictal activity. Efforts were made not to mistake artifacts for epileptic activity. Interictal epileptic activity was defined as unambiguous epileptiform potentials (EPs) characterized by single or multiple spikes followed by a slow wave and clearly interrupting background activity with respect to amplitude and frequency (Figs. 4B and 5). EPs were previously described after TeT injections

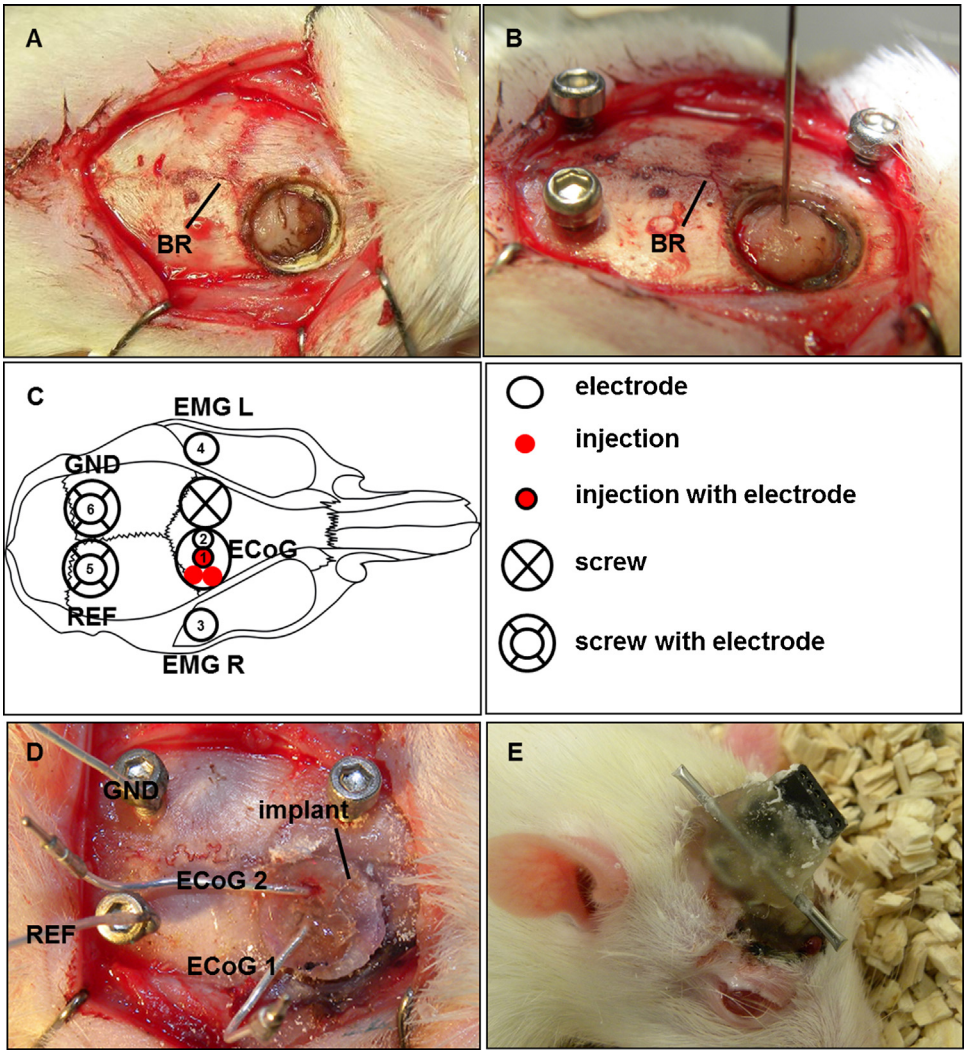

Fig. 1. Combined triple tetanus toxin injections and cobalt(II) chloride application. (A) View on the burr hole on the right frontal cranium next to the bregma (BR). The meninges were removed with a dural hook. (B) Tetanus toxin injection. (C) Positions of the six electrodes, placed in the right motorcortex (electrocorticogram, ECoG), left and right temporal muscle (electromyogram, EMG R/L) or adjacent to a screw for reference (REF) and grounding (GND). (D) Electrode placement after cobalt(II) chloride application and implant insertion. (E) Electrode fixation in plug connector after surgery.

[21,23]. After identification, EPs were counted. By definition, ictal seizure patterns consisted of runs of repetitive spike-waves or rhythmic activity with a dynamic evolution in frequency and topography (Figs. 3 and 4A).

For histology animals were anesthetized intraperitoneally with ketamine, 100 mg/kg, xylazine 5 mg/kg, atropine 0.1 mg/kg in 0.9% NaCl and transcardial perfused for 5 min with artificial cerebrospinal fluid (ACSF: 125 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $C_6H_{12}O_6$, 2 mM $CaCl_2$, 1 mM $MgCl_2$), thereafter for 10 min with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.3. Immediately after surgical removal the brain specimen were cut perpendicular to the rostro-caudal axis into sections of 50 μm (vibratome VT 1000S, Leica, Bensheim, Germany). Afterwards sections were immersion-stained for 10 min in 0.1% cresyl violet, dehydrated in increasing concentrations of ethanol, xylol and coverslipped (Immu-Mount, Thermo-Shandon, Dreieich, Germany), for details see ref. [24]. In histological sections the TeT injected rat brains, even those with multiple injections, did not show any morphological lesion, despite the opened scull and perforated dura mater (data not shown), which has been described by multiple authors [17,18,21–23,25,26]. In contrast animals treated with neocortical $CoCl_2$ showed an extended necrosis (Fig. 6).

2.3. Statistics

Assuming that there is no normal distribution in the amount of EPs/h, a Mann–Whitney *U* test was performed to compare the effect of VPA and NaCl. A *p*-value less than 0.05 was considered significant.

2.4. Histology

After decapitation, the brain was transferred to 4% paraformaldehyde in 0.4 M phosphate buffer, pH 7.4. Sections (70 μm) were prepared with a vibratome (Leica VT 1000S) for Nissl staining (microscope: Axioplan2, software: AxioVision Rel 4.8 Carl Zeiss, Oberkochen, Germany).

2.5. Drugs

Buprenorphine hydrochloride (Temgesic®, Reckitt Benckiser Healthcare Ltd., Hull, United Kingdom); cobalt(II) chloride $6H_2O$, Evans blue (Sigma–Aldrich, Steinheim, Germany); isoflurane (Forene®, Abbott GmbH and Co. KG, Wiesbaden, Germany); 0.9% NaCl and VPA (10%, w/w each) PCL-implants (Freiburg Materials Research Center FMF, Albert-Ludwigs University, Freiburg,

Germany); tetanus toxin (Quadratech Diagnostics Ltd., Epsom, Surrey, U.K.).

3. Results

3.1. Recordings in rats without injection

In 10 animals ECoG electrodes were implanted only to record physiological baseline ECoG. Recorded activity included a basic rhythm consisting of alpha, beta, theta, and delta waves with amplitudes of about 200 μV (Fig. 2). In contrast to incidental spiking in the control animals reported by Nilsen et al. [17], no epileptiform activity was observed.

3.2. Recordings in rats with single injection of 50–200 ng TeT

In five animals a single dose of 50 ng TeT was intracortically injected into the primary motor cortex of the right hemisphere through a borehole; five animals served as controls and received the same amount of saline (0.9%) instead. The NaCl-injected animals did not show any difference in their behavior or their ECoG and EMG recordings compared with the rats without any injection. In 10 animals we added Evans blue to the TeT injections and saw the intracortical diffusion of the toxin adjacent to the injection site macroscopically. In further 10 animals higher doses up to 200 ng per single injection were administered. Surprisingly, the ECoG and EMGs were not different from the control animals; in particular no constant epileptiform activity was seen. However, there was one single incidental seizure in one animal recorded on the 46th postoperative day (Fig. 3). The seizure started with facial twitching, followed by clonic movements of the upper and lower limbs. The corresponding rhythmic spike-waves in the ECoG terminated at the same time point, when the 81 s period of the seizure also stopped.

3.3. Recordings in rats with triple injections of 50 ng TeT

Eight animals were triple injected intracortically with 50 ng TeT over three boreholes; another animal was triple injected with saline (0.9%). Three of the eight animals with triple TeT injections died shortly after surgery.

Only one of the remaining five triple TeT-injected animals had recurrent seizures (Fig. 4A). These seizures started on the second postoperative day and lasted until the spontaneous death on the 10th day. The majority of these seizures manifested clinically with clonic movements, including the head and (left) forelimb. There were also some subclinical seizure patterns. In the ECoG and EMG, repetitive spike-waves with increasing frequency were observed. Another animal had a single subclinical seizure, as seen by runs of repetitive spike-waves in the ECoG. Two other animals, of which one had many EPs, displayed only single clonic jerks, which appeared as an involuntary head movement and followed single spike-waves (Fig. 4B). The last animal with triple TeT injections did not develop any epileptiform activity.

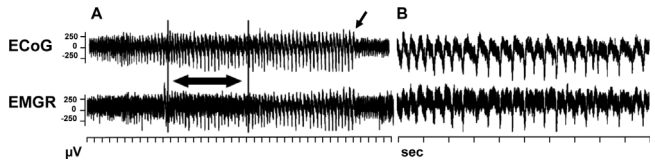

Fig. 3. Electrocorticogram (ECoG) and electromyogram (EMG) of the sole recorded seizure after single tetanus toxin injection. (A) Rhythmic spike-waves with evolving frequency and amplitude. The termination of this seizure pattern in the ECoG (inclined arrow) coincided with the termination of the tonic–clonic seizure. The high ECoG amplitudes transmitted their signal into the EMG electrodes. (B) Expanded view for the marked range from (A).

3.4. Recordings in rats with triple injections of 50 ng TeT and topically administered $CoCl_2$

Six animals with triple 50 ng TeT injections additionally received 20 mg $CoCl_2$ that was placed topically on the neocortical surface. In three of these animals, a VPA-containing PCL-implant was subsequently implanted with contact to the cortical surface. The remaining three animals served as controls; here a sham PCL-implant with NaCl was inserted. Three animals – two with NaCl-implants and one with a VPA-implant – died during the first night after surgery. All remaining animals showed EPs in the ECoG recordings (Fig. 5).

In the NaCl-implant animal, EPs occurred after three days and increased in frequency until the second week, when the frequency amounted to 271.4 EPs/h. After three weeks the EP frequency declined and finally vanished after six weeks. The EP frequency of the two remaining VPA-implant animals peaked (30 and 87 EPs/h) during the first days, then declined gradually until the EPs disappeared after three weeks. In contrast to sole triple TeT injections, triple TeT injections plus $CoCl_2$ treatment resulted in severe necrosis, spreading from the cortical M1 and M2 region to the basal ganglia (Fig. 6). The contralateral hemisphere was not affected. Because of the high mortality in the group with additional 20 mg $CoCl_2$, the dose was reduced to 10 mg.

Eight animals were triple injected with 50 ng TeT and 10 mg $CoCl_2$ was placed neocortically. Thereafter, five animals received sham NaCl-implants and three received VPA-implants. None of these animals died shortly after operation, and all but one (VPA-implant) developed EPs (Fig. 5). The NaCl-implanted animals showed EPs after the first days and the EP frequency climaxed during or after the second week (9.6–35.0 EPs/h), then gradually declined. In the two VPA-treated animals with EPs, the EP frequency also peaked after few days (341.3 or 15.7 EPs/h after one week, respectively), but already declined during the second week. The last VPA-treated animal did not show any EPs at all.

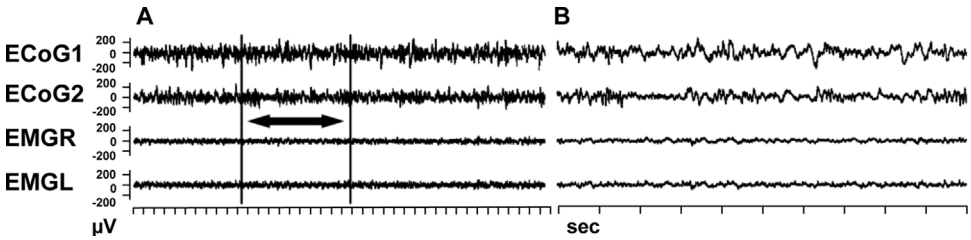

Fig. 2. Electrocorticogram (ECoG) and electromyogram (EMG) from an animal without injection and no epileptic activity. (A) ECoG electrode 1 (area M1 right hemisphere) and 2 (medial to 1) show physiological activity predominantly in the theta range. The EMGs did not show any activity of the left (L) or right (R) temporal muscle. (B) Expanded view for the marked range from A). Amplitudes shown on the *y*-axis in microvolt (μV). Timescale shown on the *x*-axis in seconds (sec).

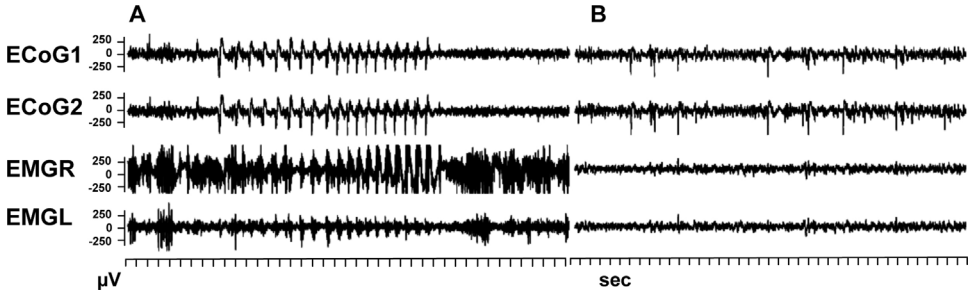

Fig. 4. Electrocorticogram (ECoG) and electromyogram (EMG) recordings from seizures and interictal spike-waves after triple tetanus toxin injections. (A) tonic–clonic seizure as presented by repetitive spike-waves with increasing frequency. (B) Interictal spike-waves in the ECoG, which were not followed by contractions of the temporal muscle in the EMG.

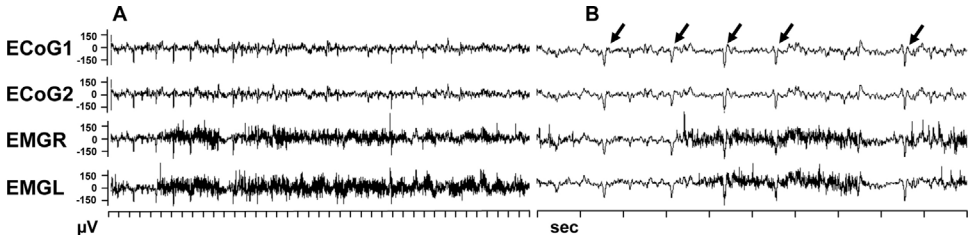

Fig. 5. Spike-waves after triple injections of 50 ng tetanus toxin and cobalt(II) chloride application on the neocortical surface. (A) The double approach produced consistent interictal spike-waves in all (except for one VPA-treated) animals but no seizures. (B) Expanded view of interictal spike-waves (arrows).

Performing a two-way analysis of variance (factor I: 20 or 10 mg $CoCl_2$; factor II: week 1 or 2), no hints were obtained indicating that results on EP frequency differ between 20 or 10 mg treated animals (factor I: $p = 0.8127$). Therefore the results of 20 or 10 mg $CoCl_2$-treated animals could be pooled together. There was, however, a high interindividual inconsistence in the triple TeT-injected plus $CoCl_2$-treated animals regarding the amount of EPs/h. The EP frequencies after the first week in the NaCl-implanted animals (mean: 10.5 EPs/h, standard error of the mean [SEM] 4.8; $n = 6$; median: 6.4 EPs/h) and VPA-implanted animals (mean: 71.6 EPs/h, SEM 67.5; $n = 5$; median: 4.6 EPs/h)), therefore, did not differ significantly, though the mean EP frequency of VPA-implant animals was higher than for NaCl-implant animals. The mean and median EP frequency after the second week was, however, significantly higher ($p = 0.0303$) in the NaCl-implant animals (mean: 53.5 EPs/h, SEM 34.9; median: 22.0 EPs/h) than in the VPA-implant (mean: 4.8 EPs/h, SEM 2.2; median: 6.7 EPs/h) animals (Fig. 7A).

The time course of EP frequencies were similar within each implant group: The EP frequency of the NaCl-implant animals increased in the second week, while the VPA-implant animals showed a reduction in their EP frequency in the second week. Comparison between the mean increase in EPs/h of the NaCl-implant animals (+42.9, SEM 32.7) and the mean decrease in EPs/h of the VPA-implant animals (−66.8, SEM 65.9) resulted in a significant difference ($p = 0.0087$) between the rates (Fig. 7B). This was also true for the 10 mg $CoCl_2$ treated animals alone (5 NaCl-implanted animals *vs.* 3 VPA-implanted animals; Fig. 7C).

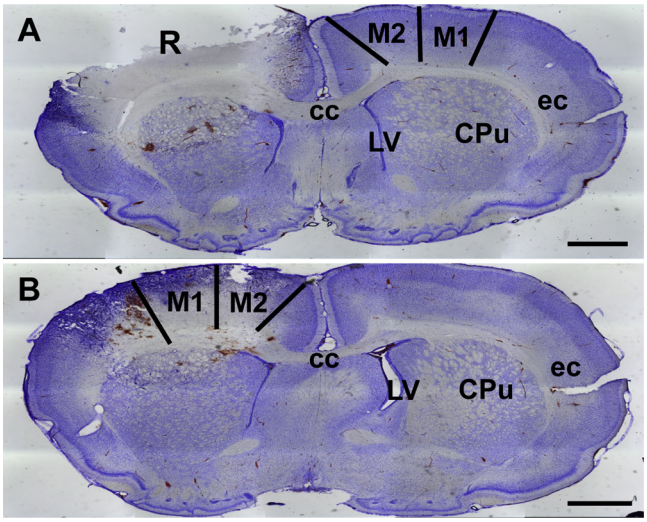

Fig. 6. Nissl-stained histology of the neocortex after triple injections of 50 ng tetanus toxin and application of 20 mg cobalt(II) chloride. Necrosis on the application site (R, right), but no affection of the contralateral side. CC: corpus callosum; CP: caudate putamen; EC: external capsule; LV: lateral ventricle; M1: primary motor cortex; M2: secondary motor cortex. Scale bars 1 mm.

4. Discussion

The aim of our study was to establish an animal model for neocortical epilepsy and to test whether a neocortically placed PCL-implant loaded with an AED would reduce seizure frequency and epileptic ECoG activity. According to the literature, the rat TeT epilepsy model is an appropriate model for chronic neocortical epilepsy [25]. In particular following the publications of Nilsen et al. [17,18], we saw the chance to establish a model where ictal and interictal ECoG activity could easily be quantified.

4.1. Single injection of TeT

In our hands the model proved to be most difficult in producing stable seizure frequencies or epileptiform ECoG activities. Despite using the same rat strain, TeT source, injection locus and ECoG electrode positioning combined with temporal muscle EMG recordings as Nilsen et al. [17], we were not able to reproduce

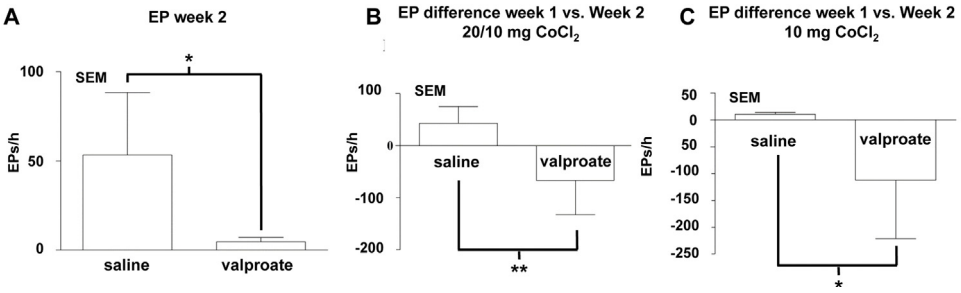

Fig. 7. Efficacy of VPA-loaded polymer-implants in reducing the frequency of epileptiform potentials (EPs). (A) The EP frequency after the second week was lower in VPA-treated ($n = 5$) animals than in saline-treated ($n = 6$) animals (20 or 10 mg cobalt(II) chloride; $CoCl_2$). (B) The increase or decrease of EPs between the first and second week were compared. Whereas saline-implanted animals ($n = 6$) showed an increase of EPs/h, VPA-implanted rats ($n = 5$) showed a decrease. (C) The increase or decrease of EPs differed also between saline-treated ($n = 5$) and VPA-treated ($n = 3$) animals without the data from the animals with 20 mg $CoCl_2$. Significant differences are indicated by asterisks: $^{*}p < 0.05$, $^{**}p < 0.01$; SEM: standard error of the mean.

their results. After confirmation of the proper injection site with Evans blue and increasing the dose per single injection up to fourfold without any relevant effect, we also tested the efficacy of the used TeT batch *in vitro*: The toxin clearly reduced the exocytotic release of ^{3}H-glutamate [27] and of ^{3}H-GABA [28] in both rat and human neocortical synaptosomes previously loaded with the corresponding transmitter.

Nilsen et al. [17] defined epileptic activity as an abrupt change of background ECoG activity, followed by a burst of fast activity evolving into rhythmic spike discharges with higher amplitudes. Spike-waves were not described (though observed previously after TeT injection; [21,23]). We were not able to reproduce these features in ECoG activity between our controls and the single-dose, 50–200 ng TeT injected rats. We observed one single tonic–clonic seizure in only one of 25 single TeT-injected rats, whose ECoG during the seizure was unambiguously different to the description of epileptic activity of Nilsen et al. [17]. Barkmeier and colleagues described also in two publications that seizures were extremely rare [29,30].

Consecutively, we were also not able to reproduce the differences in the power spectra derived from Fast Fourier Transformation of ECoG activity, which should show power elevation in the 5–20 Hz spectrum [17]. A power elevation in the 5–20 Hz spectrum was seen in control and in injected animals.

In the beginning of our experiments the frequency and amplitude of the EMG activities seldom differed from those of the ECoG recordings. By transposing the grounding and reference electrode intraosseously in the scull, we could improve the ECoG signal (compare Figs. 3 and 5). We therefore assume that, to some degree, activity recorded in the ECoG channels was actually originating in muscles and registered by both EMG and ECoG electrodes. Therefore, care has to be taken, that muscle activity is not mistaken with ictal activity in the ECoG.

4.2. Triple injection of TeT

Because we were not successful with single TeT injections, even when administering higher doses, we enlarged the injection surface area with a triple injection of 50 ng TeT over three burr holes. This enabled us to produce visible seizures and differences in the ECoG activity between TeT-injected animals and controls. Instead of a high frequency activity [17], we observed clear-cut interictal spike-waves. The rare seizures were characterized by tonic–clonic movements of the extremities and by runs of unambiguous repetitive spike-waves in ECoG recordings. Sometimes clonic jerks following single interictal discharges were observed. In the control group, where the animals received a triple-NaCl-injection, none of the animals showed EPs, therefore we assume that the TeT but not the injection-trauma alone was responsible for the EPs. However, the effect on interictal epileptic activity and seizures after triple injections of TeT varied immensely, and such a high variability prevented the final establishment of an animal model of neocortical epilepsy for the development of new treatment strategies. Therefore we decided to use $CoCl_2$ in addition to TeT.

4.3. Triple injection of TeT plus $CoCl_2$

It was shown that (heavy) metals can produce epileptic foci (see for instance [31–33]). In particular cobalt has been widely utilized in rodent epilepsy models. The cellular mechanism of cobalt-induced epilepsy is not known precisely and many possible explanations for its proconvulsive properties have been proposed: damage of inhibitory neurons [34,35], effects on postsynaptic receptors and glutamate uptake [36,37], and activity-dependent facilitation of gap junctions [38]. Eder et al. [39] described seizures induced by intrahippocampal implantation of cobalt. Tamargo et al. [40] applied 20 mg $CoCl_2$ to the rat neocortex, resulting in epileptic spikes in half of the animals, but observed an unspecified high number of rats which died during status epilepticus. Chang et al. [41] showed that implantation of a cobalt wire into the motor cortex of rats produced subacute seizures that were observable for three weeks.

However, despite the fact that these animals developed a local necrosis in the injured area, the stable EP activity decreased after four to six weeks, what was also described by Liang et al. [22]. Unfortunately there is no good explanation why the epileptic activity decreases, since other epilepsy models with morphological lesions, as the hippocampus (*e.g.* applied kainiate or pilocarpin), showed epileptic activity over a nearly unlimited time. An answer could be, that the structure of a six-neuronal-layer-thick neocortex has better antiepileptic repair mechanisms then a three-layered hippocampus. This is supported by the fact, that even TeT injections in hippocampus show EPs for month to years [21,23,42,43].

In the present study, we did not produce overt seizures with the additional $CoCl_2$ treatment. In parallel experiments, however, we used a larger TeT dose (225 ng), a medium $CoCl_2$ dose (15 mg) and also implanted NaCl- or VPA-implants [44]. Then, the animals developed reliable seizures, but also had a high mortality rate, so that quantitative evaluation of ECoG activity was not possible [44]. The $CoCl_2$-implanted animals in the present study, however, showed a stable activity of typical EPs, which enabled their quantification. We also observed a high mortality rate among the animals treated with 20 mg $CoCl_2$ and therefore decided to reduce the dose to 10 mg. None of the animals treated with 10 mg $CoCl_2$ died, but all except of one (treated with local VPA) developed stable EPs.

The stable EP activity upon triple TeT-injections and additional $CoCl_2$ enabled us to test a local VPA treatment, an antiepileptic drug commonly used in therapy of focal epilepsy. We were able to show a reduction in the rate of EPs after application of VPA directly on the epileptic focus. We hypothesize therefore that local administration of VPA might positively influence not only over-all survival [44], but also focal epilepsy. However, the rate of EPs was highest during the first two weeks. Therefore we compared the effects of VPA and NaCl polymer implants as an elevation or reduction of the frequency of EPs in this stretch of two weeks, occurring seven and 14 days after the implantation of VPA or NaCl, occurring within one week before and after the implantation operation. The measurements and operations were carried out before the forth week, since we expected and experienced a drop of the frequency of the EPs after this time point.

We admit that in the present study no animals with TeT injections plus $CoCl_2$ application were studied *without* PCL implant. However, the fabrication of VPA PCL implants was described previously [20]. We assume no further influence of the biodegradable PCL implants themselves or of their minimal NaCl load. The improbable role of the PCL implants themselves in epileptogenesis (or its prevention), however, has not (yet) been examined systematically.

It was shown by Kammerer et al. [20] in superfusion experiments that the utilized VPA-containing PCL-implants release about 88% of their VPA content *in vitro* already within the first four days; however, the course of the spiking epileptogenic focus seemed to be affected over a longer period *in vivo*. In our experiments the EPs frequency only slowly diminished over a two weeks period in animals with VPA-implants. A possible explanation for this could be that the release of VPA out of the polymer matrix might be slower under *in vivo* conditions (only one side of the polymer in contact with the cortical surface, no steady perfusion) than in an *in vitro* superfusion chamber.

5. Conclusions

After establishing a rat model with a stable interictal activity upon triple TeT-injections and additional $CoCl_2$, we could prove the in principle antiepileptic effect of local AED treatment. We plan further experiments to show its beneficial effect also on ictal activity including clinically relevant seizures.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgment

We gratefully thank Miriam Kammerer for her technical support.

References

[1] Picot MC, Baldy-Moulinier M, Daures JP, Dujols P, Crespel A. The prevalence of epilepsy and pharmacoresistant epilepsy in adults: a population-based study in a Western European country. Epilepsia 2008;49:1230–8.
[2] Sander JW, Shorvon SD. Epidemiology of the epilepsies. J Neurol Neurosurg Psychiatry 1996;61:433–43.
[3] Arroyo S, Brodie MJ, Avanzini G, Baumgartner C, Chiron C, Dulac O, et al. Is refractory epilepsy preventable? Epilepsia 2002;43:437–44.
[4] Sander JW. Some aspects of prognosis in the epilepsies: a review. Epilepsia 1993;34:1007–16.
[5] Wirrell EC. Predicting pharmacoresistance in pediatric epilepsy. Epilepsia 2013;54(Suppl. 2):19–22.
[6] Wiebe S, Blume WT, Girvin JP, Eliasziw M, Effectiveness and Efficiency of Surgery for Temporal Lobe Epilepsy Study Group. A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 2001;345:311–8.
[7] Langer RS, Peppas NA. Present and future applications of biomaterials in controlled drug delivery systems. Biomaterials 1981;2:201–14.
[8] Mak M, Fung L, Strasser JF, Saltzman WM. Distribution of drugs following controlled delivery to the brain interstitium. J Neurooncol 1995;26:91–102.
[9] Lazarowski A, Czornyj L, Lubienieki F, Girardi E, Vazquez S, D'Giano C. ABC transporters during epilepsy and mechanisms underlying multidrug resistance in refractory epilepsy. Epilepsia 2007;48(Suppl. 5):140–9.
[10] Loscher W. Drug transporters in the epileptic brain. Epilepsia 2007;48 (Suppl. 1):8–13.
[11] Bennewitz MF, Saltzman WM. Nanotechnology for delivery of drugs to the brain for epilepsy. Neurotherapeutics 2009;6:323–36.
[12] Menei P, Montero-Menei C, Venier MC, Benoit JP. Drug delivery into the brain using poly(lactide-co-glycolide) microspheres. Expert Opin Drug Deliv 2005;2:363–76.
[13] Boison D. Adenosine-based cell therapy approaches for pharmacoresistant epilepsies. Neurodegener Dis 2007;4:28–33.
[14] Kokaia M, Aebischer P, Elmer E, Bengzon J, Kalen P, Kokaia Z, et al. Seizure suppression in kindling epilepsy by intracerebral implants of GABA- but not by noradrenaline-releasing polymer matrices. Exp Brain Res 1994;100: 385–94.
[15] Serralta A, Barcia JA, Ortiz P, Duran C, Hernandez ME, Alos M. Effect of intracerebroventricular continuous infusion of valproic acid versus single i.p. and i.c.v. injections in the amygdala kindling epilepsy model. Epilepsy Res 2006;70:15–26.
[16] Stein AG, Eder HG, Blum DE, Drachev A, Fisher RS. An automated drug delivery system for focal epilepsy. Epilepsy Res 2000;39:103–14.
[17] Nilsen KE, Walker MC, Cock HR. Characterization of the tetanus toxin model of refractory focal neocortical epilepsy in the rat. Epilepsia 2005; 46:179–87.
[18] Nilsen KE, Kelso AR, Cock HR. Antiepileptic effect of gap-junction blockers in a rat model of refractory focal cortical epilepsy. Epilepsia 2006;47:1169–75.
[19] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. Burlington, U.S.A.: Elsevier Inc.; 2007.
[20] Kammerer M, Fabritius M, Carvalho C, Mulhaupt R, Feuerstein TJ, Trittler R. Valproate release from polycaprolactone implants prepared by 3D-bioplotting. Pharmazie 2011;66:511–6.
[21] Brener K, Amitai Y, Jefferys JG, Gutnick MJ. Chronic epileptic foci in neocortex: in vivo and in vitro effects of tetanus toxin. Eur J Neurosci 1991;3:47–54.
[22] Liang F, Le LD, Jones EG. Reciprocal up- and down-regulation of BDNF mRNA in tetanus toxin-induced epileptic focus and inhibitory surround in cerebral cortex. Cereb Cortex 1998;8:481–91.
[23] Finnerty GT, Jefferys JG. Investigation of the neuronal aggregate generating seizures in the rat tetanus toxin model of epilepsy. J Neurophysiol 2002;88:2919–27.
[24] Freiman TM, Eismann-Schweimler J, Frotscher M. Granule cell dispersion in temporal lobe epilepsy is associated with changes in dendritic orientation and spine distribution. Exp Neurol 2011;229:332–8.
[25] Benke TA, Swann J. The tetanus toxin model of chronic epilepsy. Adv Exp Med Biol 2004;548:226–38.
[26] Mainardi M, Pietrasanta M, Vannini E, Rossetto O, Caleo M. Tetanus neurotoxin-induced epilepsy in mouse visual cortex. Epilepsia 2012;53:e132–6.
[27] Kammerer M, Brawek B, Freiman TM, Jackisch R, Feuerstein TJ. Effects of antiepileptic drugs on glutamate release from rat and human neocortical synaptosomes. Naunyn Schmiedebergs Arch Pharmacol 2011;383:531–42.
[28] Kammerer M, Rassner MP, Freiman TM, Feuerstein TJ. Effects of antiepileptic drugs on GABA release from rat and human neocortical synaptosomes. Naunyn Schmiedebergs Arch Pharmacol 2011;384:47–57.
[29] Barkmeier DT, Loeb JA. An animal model to study the clinical significance of interictal spiking. Clin EEG Neurosci 2009;40:234–8.
[30] Barkmeier DT, Senador D, Leclercq K, Pai D, Hua J, Boutros NN, et al. Electrical, molecular and behavioral effects of interictal spiking in the rat. Neurobiol Dis 2012;47:92–101.
[31] Bostantjopoulou S, Katsarou Z, Milonas I, Taskos N, Logothetis J. Focal experimental epilepsy in rabbits. Funct Neurol 1990;5:127–33.
[32] Craig CR, Colasanti BK. GABA receptors, lipids, and gangliosides in cobalt epileptic focus. Adv Neurol 1986;44:379–91.
[33] Willmore LJ, Sypert GW, Munson JB. Recurrent seizures induced by cortical iron injection: a model of posttraumatic epilepsy. Ann Neurol 1978;4: 329–36.
[34] Esclapez M, Trottier S. Changes in GABA-immunoreactive cell density during motor focal epilepsy induced by cobalt in the rat. Exp Brain Res 1989;76: 369–85.
[35] Sugaya E, Ishige A, Sediguchi K, Yuzurihara M, Iizuka S, Sugimoto A, et al. Damage of hippocampal neurons caused by cobalt focus in the cerebral cortex of rats. Brain Res 1988;459:196–9.
[36] Gerber U, Gahwiler BH. Cobalt blocks postsynaptic responses induced by neurotransmitters in the hippocampus in vitro. Neurosci Lett 1991;134:53–6.
[37] Mafra RA, Araujo DA, Beirao PS, Cruz JS. Glutamate transport in rat cerebellar granule cells is impaired by inorganic epileptogenic agents. Neurosci Lett 2001;310:85–8.
[38] He J, Hsiang HL, Wu C, Mylvagnanam S, Carlen PL, Zhang L. Cellular mechanisms of cobalt-induced hippocampal epileptiform discharges. Epilepsia 2009;50:99–115.

[39] Eder HG, Stein A, Fisher RS. Interictal and ictal activity in the rat cobalt/pilocarpine model of epilepsy decreased by local perfusion of diazepam. Epilepsy Res 1997;29:17–24.
[40] Tamargo RJ, Rossell LA, Kossoff EH, Tyler BM, Ewend MG, Aryanpur JJ. The intracerebral administration of phenytoin using controlled-release polymers reduces experimental seizures in rats. Epilepsy Res 2002;48:145–55.
[41] Chang JH, Yang XF, Zempel JM, Rothman SM. The unilateral cobalt wire model of neocortical epilepsy: a method of producing subacute focal seizures in rodents. Epilepsy Res 2004;61:153–60.
[42] Jiruska P, Finnerty GT, Powell AD, Lofti N, Cmejla R, Jefferys JG. Epileptic high-frequency network activity in a model of non-lesional temporal lobe epilepsy. Brain 2010;133:1380–90.
[43] Empson RM, Amitai Y, Jefferys JG, Gutnick MJ. Injection of tetanus toxin into the neocortex elicits persistent epileptiform activity but only transient impairment of GABA release. Neuroscience 1993;57:235–9.
[44] Altenmuller DM, Hebel JM, Rassner MP, Volz S, Freiman TM, Feuerstein TJ, et al. Locally applied valproate enhances survival in rats after neocortical treatment with tetanus toxin and cobalt chloride. Biomed Res Int 2013;2013:497485.